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Gene flow and immigration rate in an island population of great tits

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Abstract

It is generally recognized that immigration and gene flow cannot be equated, but few detailed quantitative comparisons of these processes have been made over the entire lifetime of individual animals. We analyzed data collected in a longterm study of an island population of great tits *Parus major*, and tested two assumptions frequently made in population genetic studies. (1) The assumption that there is no difference in reproductive output between immigrant and resident breeding birds was refuted for females but not for males. Lifetime production of local recruits (LRS) was reduced by 37% in immigrant females, because female immigrants tended to leave the island after breeding, and thus reproduced for fewer years. Female LRS was density dependent, but the effect of density was independent of status (resident/immigrant). (2) The assumption that mating was random with respect to status was also refuted: assortative mating was found, even when temporal and spatial aggregation of immigrants was controlled for. Thus both assumptions were refuted, and gene flow was lower than immigration rate.

Introduction

Patterns and intensity of gene flow, together with mutation rate, genetic drift and natural selection, determine the genetic structure of populations (Ehrlich and

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Raven 1969; Endler, 1977). Hence gene flow plays an important role in population genetic processes such as local adaptation and speciation. The effect of gene flow on population genetic structure has been the subject of a large body of theoretical work (reviewed by Slatkin 1985), but empirical aspects of gene flow are less developed.

Gene flow is strongly related to dispersal, and immigration rates as well as dispersal distances have been used to obtain direct estimates of gene flow (Rockwell and Cooke, 1977; Barrowclough, 1980; Slatkin, 1985). However, immigration (and hence dispersal) in the demographic sense cannot be equated with gene flow (e.g., Ehrlich and Raven, 1969; Endler, 1977; Rockwell and Barrowclough, 1987) because several factors may affect the incorporation of immigrant genes in a population. Although this point is generally accepted, lack of demographic information often leaves no other option than to infer gene flow from the existence of a breeding pair which includes an immigrant. Such studies implicitly assume there is no difference in reproductive output between immigrants and residents in a population (or, depending on the method used, that reproductive output is independent of dispersal distance).

The incorporation of alleles carried by immigrants into an existing population also depends on the mating pattern. For example, assortative mating with respect to status (immigrant/resident) reduces incorporation of immigrant genes in the population. Genetic models usually assume random mating, and non random mating results in serious complication of such models (see Rockwell and Barrowclough, 1987, for an overview). Unfortunately, studies that tested the assumption that mating is random with respect to immigration status are rare, despite their relevance for population genetic models.

In this paper we describe the rate of immigration into the breeding population of great tits *Parus major* on Vlieland, an island in the Dutch Waddensea (Kluyver, 1971; Verhulst, 1995) where great tits have been monitored continuously since 1955. Due to the limited amount of woodland on the island, and the characteristic of great tits to breed in nest boxes when they are available, we are able to ring practically all nestlings in this population. As a result, immigrant and resident breeding birds can be distinguished (van Tienderen and van Noordwijk, 1988). Deleterious effects of inbreeding have been demonstrated in this population (van Noordwijk and Scharloo, 1981), making further knowledge of gene flow in this population of importance. We used the data from this population to test two assumptions frequently made in population genetic studies. (1) The assumption that there is no difference in reproductive output between immigrant and resident breeding birds. (2) The assumption that mating was random with respect to status (resident/immigrant).

Study area and methods

The surface area of Vlieland (53.17° N, 5.03° E) is 3258 ha. Approximately 1500 ha is taken up by the Vliehors, a sandy area with very little vegetation on the

west side of the island. Of the remaining 1750 ha, the total area covered with woodland is 295 ha (9%). There are five separate woodlands on Vlieland that differ in size and vegetation (Kluyver, 1971). The land between the woodlands consists mainly of dunes. There is one relatively large woodland, the village wood (215 ha), and four small woodlands, which together are usually referred to as the west.

Standard fieldwork in the breeding season starts with regular inspection of the nest boxes, usually once a week. During these inspections the following parameters are recorded: the state of the nest (during nest-building), the number of eggs and the number of young. Nestboxes were inspected regularly around the expected time of hatching to establish the hatching date. When a female was found incubating she was identified by her colour rings, and parents were captured with spring traps when the young were approximately eight days old. Captured birds were aged following Svensson (1984). The young were ringed at the same time. After fledging, remains of nests were removed, and searched for unhatched eggs, dead nestlings and their rings.

Immigrants were defined as birds not ringed as nestling on Vlieland. Occasionally Great Tits breed in nestboxes that are unknown to us, or in natural cavities, in which case the nestlings remain unringed. However, this concerns a very small part of the population, and it can therefore safely be assumed that the 'not ringed as nestling' criterion yelds reliable estimates of the proportion of immigrants.

Great Tits are monogamous, but extra-pair fertilizations occur (Blakely, 1994), although not at a very high rate. Intra-specific brood parasitism is very rare in Great Tits, if it occurs at all (Kempenaers et al., 1995; pers. obs.). It can therefore safely be assumed that females caught at the nest where chicks were ringed are the mothers, while parentage of the male is less certain.

Lifetime production of recruits (LRS) was determined by counting all local recruits a bird produced in its life. Recruits are offspring recaptured as breeding birds on Vlieland. LRS was determined for all birds that first bred in 1975–1983, and local recruits that started breeding up to 1988 were included (great tits usually start breeding as yearlings). This period was selected because in these years no experiments were carried out in this population (Tinbergen et al., 1985; Verhulst, 1995). The distribution of the number of recruits resembles a Poisson-distribution (van Balen et al., 1987), and therefore LRS-data were analyzed by fitting a logarithmic model with Poisson error distribution using GLIM (NAG 1987; Crawley, 1993). Suvival data were analyzed with logistic regression (Sokal and Rohlf, 1994). All tests were two-tailed.

Results

Immigration rate

The proportion of immigrants among new breeders decreased up to 1962, and has been relatively stable since then (Fig. 1). The proportion of immigrants among male and female new breeders was significantly correlated (years 1956–92,

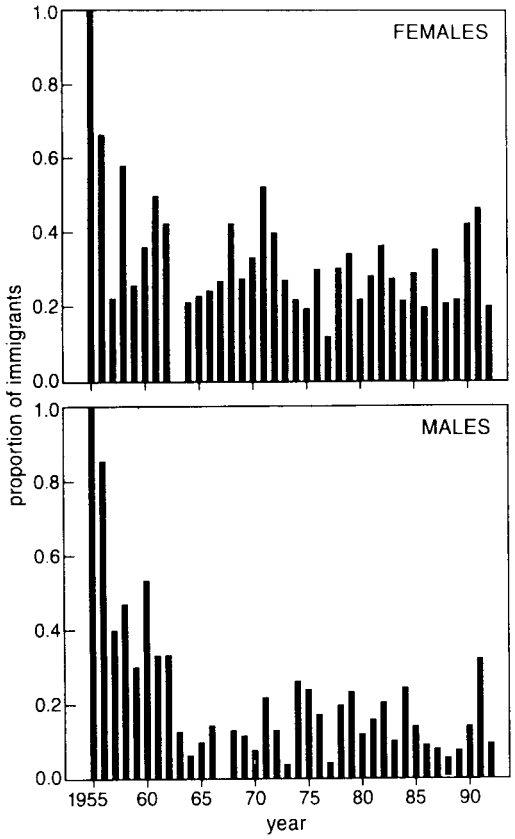

Fig. 1. Proportion of immigrants (birds not ringed as nestling on Vlieland) among first time breeders in the years 1955–1992. A: females, B: males.

$r_s = 0.49$, $n = 37$, $P < .005$), but the proportion of immigrants was consistently lower among males (Wilcoxon matched pairs signed ranks test, $P < .001$, $n = 37$, years 1956–92). The mean proportion of immigrants among new breeders in the years 1963–1992, (the 'stable' period), was 0.282 (s.d. = 0.106, $n = 30$) among females and 0.138 (s.d. = 0.075, $n = 30$) among males. Most new breeders were yearlings (1963–1992; males 85.3%, $n = 1326$; females 96.6%, $n = 1458$). True proportions of yearlings are even higher because some breeding birds are not identified in their first breeding season.

Fecundity

LRS was compared between resident and immigrant great tits to assess the relationship between immigration rate and gene flow into the population. Only

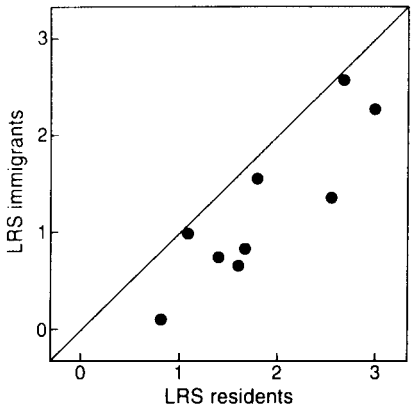

Fig. 2. Lifetime production of local recruits (LRS) of immigrant and resident females. Each data point represents one cohort of females born in the years 1974–1982.

birds first captured as yearlings were included. There were 455 males from nine cohorts available for this analysis which produced a total of 722 local recruits. Of the breeding males 15% (69/455) were immigrants, and 13% (97/722) of the recruits had an immigrant father. There is no significant difference between these proportions ($\chi^2 = 0.7$, d.f. = 1, $P = 0.4$), suggesting that for males immigration rate was equal to gene flow. An analysis in which the LRS of immigrant and resident males was compared confirmed this result: controlling for cohort ($F_{8,445} = 2.4$, $P < .02$), there was no significant difference in LRS between resident and immigrant males ($F_{1,445} = 1.9$, n.s.).

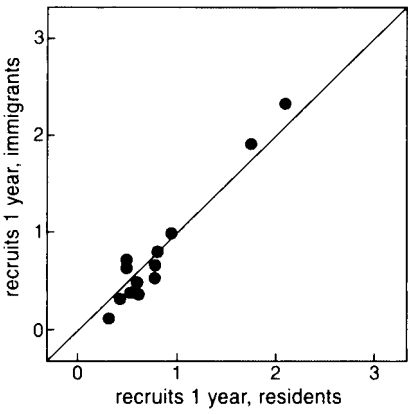

Fig. 3. The number of local recruits produced in one season by one year old immigrant and resident females that bred in the years 1975–1987.

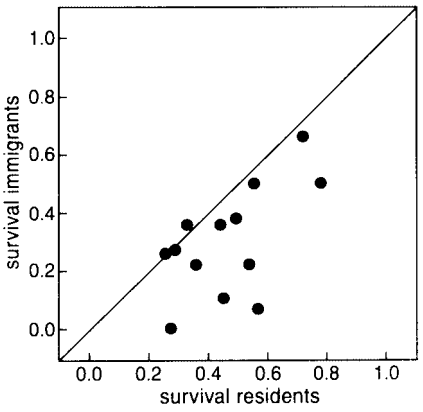

Fig. 4. Local survival of immingrant and resident one year old females that bred in the years 1975–1987.

There were 493 females from nine cohorts which produced a total of 726 local recruits. Of the breeding females 27% (133/493) were immigrants, and 20% (145/726) of the local recruits produced by these females had an immigrant mother. The proportion of recruits with an immigrant mother was significantly lower than the proportion of immigrants among the breeding females. ($\chi^2 = 8.2$, d.f. = 1, $P < 0.005$), indicating female immigration rate was higher than gene flow. An analysis in which the LRS of immigrant and resident females was compared confirmed this result (Fig. 2): controlling for cohort ($F_{8,483} = 7.2$, $P < .001$) resident females had a higher LRS than immigrant females ($F_{1,483} = 11.8$, $P < .001$). On average, LRS of immigrants was 0.6 recruits (37%) lower than the LRS of residents.

Male immigration rate was lower than female immigration rate, but the production of recruits was reduced in immigrant females, and these contrasts could have canceled each other out. However, this was not the case since among the identified parents of locally born recruits, immigrant mothers occurred at a higher frequency than immigrant fathers ($\chi^2 = 11.1$, d.f. = 1, $P < 0.001$).

The difference in LRS between immigrant and resident females could be due to a difference in longevity, a difference in productivity, or a combination. The number of recruits produced by females in their first breeding season ($n = 729$ females in the years 1975–87) did not differ between residents and immigrants (Fig. 3; $F_{1,715} = 0.9$, n.s.; controlling for year). However, in this group of females, immigrants had lower survival rates until the next breeding season than residents (Fig. 4; logistic regression: $F_{1,644} = 16.6$, $P < .001$; controlling for year $F_{12,644} = 2.9$, $P < .001$). Thus it can be concluded that the difference in LRS between resident and immigrant females was due to a difference in longevity rather than a difference in productivity.

The reduction in local survival rate of immigrant females could reflect a difference in global survival rate. Alternatively, immigrant females could be more likely to leave Vlieland after breeding. If the latter was true, immigrants should be

relatively more numerous among females that were captured outside Vlieland after breeding on Vlieland. Because of the low numbers of birds reported away from Vlieland, we used the largest possible data set to test this hypothesis. In the years 1956–91 a total of 1529 individual females have been recorded to breed on Vlieland, of which 451 (29.5%) were immigrants. Up to 11.3.1993 nine females have been captured away from Vlieland, after having been recorded as breeding birds. None of these recaptures were in a breeding season (April–August). Of these nine females, seven were immigrants (78%), which deviates significantly from what would be expected if dispersal was random with respect to status (χ^2 (with Yates' correction) = 8.0, d.f. = 1, $P < .005$).

Density dependence

The effect of status on LRS was apparently due to dispersal after breeding of immigrant females. Breeding dispersal could be density dependent, in which case gene flow could be density dependent. Therefore we investigated the relationship between density and LRS.

Mean LRS was calculated per cohort, and density was expressed as the number of pairs per 10 ha in the first year of breeding of the cohort concerned. (The density in later years cannot be included since it is in part determined by the reproductive rate in the first year, and hence no longer independent). The effect of an extra pair on the resources available per pair will depend on the number of pairs already present (Tinbergen et al. 1985), and therefore the inverse of the density was used in the analysis. LRS declined significantly with increasing density (Fig. 5; $r = .92$, $n = 9$, $P < .001$).

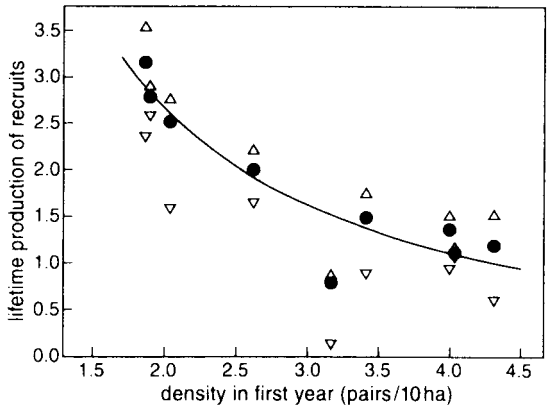

Fig. 5. Density of breeding birds and lifetime production of recruits (△ resident females, ▽ immigrant females, ● weighted overall mean). Data points represent means of cohorts that started breeding in the years 1975–1983, plotted against the density of breeding pairs in the first year of breeding. Regression line shown is for all birds ($Y = -0.45 + 6.23\ (1/X)$).

To investigate if the relationship between gene flow and immigration rate was density dependent, we tested for a correlation between (the inverse of) density and the ratio of LRS of immigrants and residents. This relationship was not significant ($r_s = 0.17$, $n = 9$, $P = 0.7$), indicating that the relationship between immigration rate and gene flow was independent of density.

Assortative mating

The first breeding attempt of all females that started breeding in the years 1963–1992 of which the partner was identified were used in the analysis ($n = 1308$). Data were analyzed with logistic regresion, because of the binomial distribution of status. Male and female status were significantly positively correlated ($F_{1,1306} = 25.5$, $P < .0001$), indicating assortative mating with respect to status. Among resident females, 11.6% ($n = 921$) had an immigrant partner, while the corresponding figure for immigrant females was 24.0% ($n = 387$). This correlation could be the consequence of spatial or temporal aggregation of immigrants. However, when we controlled for these factors by including year and woodland in the regression model, assortative mating remained significant (year: $F_{29,1273} = 2.3$, $P < .001$; woodland: $F_{4,1273} = 22.3$, $P < .001$; assortative mating: $F_{1,1273} = 4.7$, $P < .05$).

Discussion

The proportion of immigrants among new breeders on Vlieland was approximately 20%, substantially lower than the 50% reported for other great tit populations (Kluyver, 1951; van Balen, 1980; McCleery and Clobert, 1990). This is in agreement with the geographic isolation of this population (Kluyver, 1971). Nevertheless, the proportion of immigrants was still considerable and the great tits on Vlieland cannot be considered a 'small isolated population' as often considered conservation biology (Caughley, 1993).

Immigrant females, but not immigrant males, produced fewer local recruits than residents (Fig. 2). Although we tried to ring all nestlings hatched on Vlieland, a small proportion probably escaped our attention, and hence some residents were misclassified as immigrants. The true difference in LRS between immigrant and resident females will therefore be slightly larger than we estimated. We conclude therefore that for females, gene flow was lower than immigration rate, which will promote genetic differentiation between the population on Vlieland and other populations. Furthermore, it implies that the role of immigrant recruits in population dynamics is reduced as compared with resident recruits.

In a comparable study of great tits breeding in Wytham wood (UK) it was found that immigrants of both sexes produced fewer recruits per breeding attempt than residents (McCleery and Clobert, 1990), while there was no consistent difference in survival rate between immigrants and residents (Clobert et al., 1988). Although LRS of immigrants and residents was not compared directly, this strongly suggests that also in this population gene flow is lower than immigration rate.

LRS was strongly density dependent (Fig. 5), but the effect of density did not differ between the immigrant and resident females. We conclude therefore that the discrepancy between immigration rate and gene flow was independent of density. Several fitness components have been shown to covary with density in great tits, for example clutch size, fledgling recruitment and the occurrence of second clutches (Kluyver 1951, 1971; van Balen, 1973; Tinbergen et al., 1985; Verhulst, 1992). We did not investigate which components of LRS were affected by density in our study, but this clearly deserves further study.

The proportion of immigrants was higher among females, as compared to males which is in agreement with an earlier study of this population (van Tienderen and van Noordwijk, 1988). LRS was reduced in immigrant females, but, because female immigration rate was higher than male immigration rate, locally born recruits more often had immigrant mothers than immigrant fathers.

The difference between resident and immigrant females in LRS was due to a difference in the number of seasons females bred on Vlieland. Immigrant females were more likely to be recaptured away from Vlieland after breeding than resident females. Great tits tend to spend consecutive winters in the same area, even if this is not the area where they breed (P.J. Drent, unpublished). The difference in local survival rate may reflect the costs of migrating to and from an area to spend the winter, if birds suffer enhanced mortality risk during migration. Alternatively, immigrant females may be more likely to breed in another area in the next season, perhaps near the area where they spent the winter. Clobert et al. (1988) found no consistent difference between survival rates of immigrant and resident great tit females breeding in Wytham wood, but presumably the costs of travelling to another area are much lower than for the Vlieland population.

Assortative mating

Assortative mating was found with respect to status: immigrants tended to be mated to other immigrants. This was also noted in the Great Tit population breeding in Wytham Wood (UK) (Greenwood et al., 1979), although Greenwood et al. did not control for effects of spatial and temporal aggregation of immigrants. Assortative mating reduces the effective rate of immigration, and thus modifies the effect of immigrants on the genetic structure of the population.

It is not clear what factors caused assortative mating in the population on Vlieland. Assortative mating with respect to phenotypic aspects (e.g., Cooch and Beardmore, 1959), or selective pairing between familiar associates from early life (e.g., Choudhury and Black, 1994) could be involved. Alternatively, spatial aggregation of immigrants on a finer scale than we could control for statistically, could account for the observed association, or some immigrants may arrive on Vlieland already paired. Observations of pair formation are required to tease apart these possibilities. It is important to note however that the modifying effect of assortative mating arises regardless of the mechanism causing this association.

In an earlier study of the Vlieland population, van Noordwijk et al. (1985) concluded that mating was random with respect to relatedness, which contrasts with our results since residents are more closely related to each other than to immigrants. Van Noordwijk et al. used roughly the first half of the study period used in the present study, and further analysis showed that assortative mating was stronger in the second half (1983–1994) of the study period than in the first half (1964–1982; S. Verhulst, unpublished). Thus there is no conflict between our conclusions, although the cause of this temporal variation in the mating pattern remains unidentified.

Population structure

The development of molecular techniques had led to a rapid increase in the number of studies of the genetic structure of populations. These studies usually compare relatedness within and among populations using estimates of relatedness based on, for example, DNA fingerprinting (e.g., Avise, 1992; Triggs et al., 1992; Degnan, 1993; Randi and Alkon, 1994; Dias, 1994). Although molecular techniques have proven to be a powerful tool to reveal genetic population structure, these studies provide only a snapshop of the current situation. Genetic population structure is determined by a combination of genetic and demographic processes, and it is not clear what, if anything, can be inferred from a 'genetic snapshot' regarding the processes that gave rise to the structure observed (see Avise et al. (1992) for an example). When a genetic structure is present, this could be due to low levels of gene flow, but, alternatively, immigrants could in some way have a reduced offspring production (as observed in this study). This illustrates the importance of an intregration of demographic and genetic studies to understand genetic population structures.

Conclusions

We tested two assumptions fequently made in population genetic models. The assumption that reproductive output was independent of status (or dispersal) was shown to be invalid for females (Fig. 2). The habit of great tits to spend consecutive winters in the same area may be the cause of this effect, but whatever the cause, it implies that immigration rates do not provide an unbiased estimate of gene flow. The assumption that mating was random was with respect to status also shown to be invalid, which further reduces the incorporation of immigrant genes in this population.

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References

Avise, J. C. 1992. Molcular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63: 62–76.

Avise, J. C., R. T. Alisauskas, W. S. Nelson and C. D. Ankney. 1992. Matriarchal population genetic structure in an avian species with female natal philopatry. Evolution 46: 1084–1096.

Barrowclough, G. F. 1980. Gene flow, effective population sizes, and genetic variance components in birds. Evolution 34: 789–798.

Blakey, J. K. 1994. Genetic evidence for extra-pair fertilizations in a monogamous passerine, the great tit *Parus major*. Ibis 136: 457–462.

Caughley, G. 1993. Directions in conservation biology. J. Anim. Ecol. 63: 215–244.

Choudhury, S. and J. M. Black. 1994. Barnacle geese preferentially pair with familiar associates from early life. Anim. Behav. 48: 81–88.

Clobert, J., C. M. Perrins, R. H. McCleery and A. G. Gosler. 1988. Survival rate in the great tit *Parus major* in relation to sex, age and immigrant status. J. Anim. Ecol. 57: 287–306.

Cooch, F. G. and J. A. Beardmore. 1959. Assortative mating and reciprocal difference in the blue-snow complex. Nature 183: 1833–1834.

Crawley, M. J. 1993. GLIM for Ecologists. Blackwell Scientific Publications, Oxford.

Degnan, S. M. 1993. Genetic variability and population differentiation inferred from DNA fingerprinting in silvereyes (Aves: Zosteropidae). Evolution 47: 1105–1117.

Dias, P. C. 1994. Adaptation et maladaptation des mésanges bleues dans les mosaiques d'habitats méditérranéens: l'hypothese source-puits. PhD-Thesis, University of Montpellier.

Ehrlich, P. R. and P. H. Raven. 1969. Differentiation of populations. Science 165: 1228–1232.

Endler, J. A. 1977. Geographic Variation, Speciation, and Clines. Princeton University Press, New Jersey.

Greenwood, P. J., P. H. Harvey and C. M. Perrins. 1979. Mate selection in the great tit *Parus major* in relation to age, status and natal dispersal. Orn. Fenn. 56: 75–86.

Kempenaers, B., R. Pinxten and M. Eens. 1995. Intraspecific brood parasitism in two tit Parus species: occurrence and responses to experimental parasitism. J. Avian Biol. 26: 114–120.

Kluyver, H. N. 1951. The population ecology of the Great Tit *Parus m. major* L. Ardea 39: 1–135.

Kluyver, H. N. 1971. Regulation of numbers in populations of Great Tits (*Parus m. major*), pp. 507–523. Proceedings Advanced Study Institute on 'Dynamics of Numbers in Populations', Oosterbeek, and the Netherlands 7–18 September 1970. Pudoc, Wageningen.

McCleery, R. H. and J. Clobert. 1990. Differences in recruitment of young by immigrant and resident Great Tits in Wytham Wood. pp. 423–440. *In* J. Blondel et al. (Eds.), Population Biology of Passerine Birds: an Integrated Approach. Nato series G, Vol. 24. Springer-Verlag, Berlin.

Numerical Algorithms Group 1987. GLIM manual release 3.77. NAG, Oxford.

Randi, E. and P. U. Alkon. 1994. Genetic structure of Chukar (*Alectoris chukar*) populations in Israel. Auk 111: 416–426.

Rockwell, R. F., and G. F. Barrowclough. 1987. Gene flow and the genetic structure of populations. pp. 223–255. *In* T. Burke et al. (Eds.), Avian Genetics. Academic Press, London.

Rockwell, R. F., and F. Cooke. 1977. Gene flow and local adaption in a colonially nesting dimorpic bird: the lesser snow goose (*Anser caerulescens caerulescens*). Am. Nat. 111: 91–97.

Slatkin, M. 1985. Gene flow in natural populations. Ann. Rev. Ecol. Syst. 16: 393–430.

Sokal, R. and F. J. Rohlf. 1994. Biometry, 3rd edn. WH Freeman, New York.

Svensson, L. 1984. Identification Guide to European Passerines, 3rd edn. Naturhistoriska Riksmuseet, Stockholm.

Tinbergen, J. M., J. H. van Balen and H. M. van Eck. 1985. Density dependent survival in an isolated great tit population: Kluyvers data reanalysed. Ardea 73: 38–48.

Triggs, S. J., M. J. Williams, S. J. Marshall and G. K. Chambers. 1992. Genetic structure of blue duck (*Hymenolaimus malacorhynchos*) populations revealed by DNA fingerprinting. Auk 109: 80–89.

van Balen, J. H. 1973. A comparative study of the breeding ecology of the Great Tit *Parus major* in different habitats. Ardea 61: 1–93.

van Balen, J. H. 1980. Population fluctuations of the Great Tit and feeding conditions in winter. Ardea 68: 143–164.

van Balen, J. H., A. J. Van Noordwijk and J. Visser. 1987. Lifetime reproductive success and recruitment in two Great Tit populations. Ardea 75: 1–12.

van Noordwijk, A. J. and W. Scharloo. 1981. Inbreeding in an island population of the Great Tit. Evolution 35: 674–688.

van Noorkwijk, A. J., P. H. van Tienderen, G. de Jong and J. H. van Balen. 1995. Genealogical evidence for random mating in a natural population of the great tit (*Parus major* L.). Naturwissenschaften 72: 104–105.

van Tienderen, P. H. and A. J. van Noordwijk. 1988. Dispersal, kinship and inbreeding in an island population of Great Tit. J. Evol. Biol. 2: 117–137.

Verhulst, S. 1992. Effects of density, beech crop and winter feeding on survival of juvenile Great Tits; an analysis of Kluyver's removal experiment. Ardea 80: 285–292.

Verhulst, S. 1995. Reproductive decisions in Great Tits; An optimality approach. PhD-Thesis, University of Groningen.

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